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BRIEF COMMUNICATION

Permanent Slides after Detection of Starch Grains with Lugol's Solution

J. KUTÍK and K. BENEŠ

Institute of Experimental Botany, Czechoslovak Academy of Science, Praha*

Abstract. For the detection of starch in paraffin sections of FAA fixed root tips of pea and maize the Lugol's solution of suitable constitution and proper dilution was tried. JJK containing solution of arabic gum was proved as a mounting medium preserving the resulting colour. A procedure is given combining the application of JJK with alcian blue staining.

From the chemist's point of view the term starch does not denote any individual substance, but is a group name for different polyglucosanes, which commonly occur together. From the morphologist's point of view, these polyglucosanes constitute formations called starch grains and/or starch inclusions. Two cases are to be distinguished according to the course or state of starch formation: 1. a starch inclusion appears within the plastid and 2. starch predominates considerably over the remnants of the original plastid forming a starch grain. The physiologists take starch *i.e.* the starch inclusion or the starch grain as a metabolic reserve displaying considerable plasticity according to the metabolic state of the tissue. The appearance of starch is often limited to distinct portions of the tissue, *e.g.* the starch sheaths. This is the reason of our interest in starch, its localization and the regulation of its accumulation and breakdown.

The necessary prerequisite for any comparative study of starch localization and its dynamics is a suitable method of its detection resulting in permanent slides. Different techniques may be used to visualize starch (SEIDEMANN 1966, CZAJA 1969). Polarization microscopy and staining with dyes will not be discussed here. The periodic acid/Schiff (PAS) reaction is well founded chemically, but it is not limited to starch only and, moreover, the colouring of cell walls makes the evaluation of slices rather difficult. The demonstration of starch with iodine* seems most promising. Omitting here the way of handling material, two problems must be considered: the reaction proper and the maintenance of the resulting colour.

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* *Address:* Ke dvoru 15, 166 30 Praha 6, Czechoslovakia.

Several questions are open in the first case. Thus, the composition (qualitative) of the iodine containing solutions may be quite different. The most common is Lugol's solution (Lugol's iodine, jodjodkalium, abbreviated as JJK, IKI *etc.*) prepared by dissolving J in the solution of KJ in water. The J : KJ relation differs according to different prescriptions as well as the concentration of the solution as a whole. Besides, solutions lacking KJ are sometimes used. Ethanol serves as the solvent in such cases. Moreover, some ingredients like chloralhydrate or phenol are sometimes recommended as "clarifiers" or "swellers". Otherwise, factors like time, light/dark and temperature must be considered.

The second point mentioned is the effort to maintain the resulting colour. Several recommendations have been given in the literature including — in addition to starch — the staining of glycogen (*e.g.* SCHNEIDER and ZIMMERMANN 1922, SMITH *et al.* 1966, PEARSE 1968). Recently HINCHMAN (1973) tried the addition of a more or less defined amount of JJK to the aqueous mounting medium Karo sirup red label.

Taking the findings of HINCHMAN (1973) as the starting point and taking into account both groups of the problems mentioned and the way of tissue preparation omitted above, the following questions are to be solved in the present work : 1. Which is the most suitable constitution of the reagent to be applied to our material? 2. Which other medium may be used instead of JJK/Karo sirup for mounting? 3. Are the results of the detection of starch different in paraffin and in frozen sections? 4. Is it possible to keep the material unchanged for some time concerning the stainability of starch grains? 5. May the iodine reaction be combined with some cell wall staining, *e.g.* with alcian blue?

The standard material of the present study were 10 μm thick paraffin sections of root tips of pea (*Pisum sativum* L.) and maize (*Zea mays* L.). The seeds were soaked in running tap water overnight and then allowed to germinate at 25 °C in the dark in a humid atmosphere in a glass container for 2 or 3 days. The procedure was: fixation with the FAA fixative (50% ethanol 90 ml, formol 5 ml, glacial acetic acid 5 ml) overnight at lab. temperature, washing in 50% ethanol, dehydration with a tertiary butanol series and embedding into paraffine. The slides with sections were deparaffinized through xylen—ethanol series and brought to H_2O.

After some preliminary experiments, Lugol's solution as given by HINCHMAN (1973) was used. The stock solution of JJK was prepared as follows: 2 g KJ were dissolved in about 5 ml H_2O and 1 g J was added. After J got into solution, H_2O was supplied up to 100 ml. This stock solution was diluted 1 : 5 with H_2O and used within several days. This dilution appeared as the most suitable. The slices were stained 10 min at laboratory temperature in the light. Otherwise, the solutions were kept in tightly closed bottles in the dark.

The following aqueous media, either available commercially or prepared as given, were tested: Aquamount (E. Gurr), Hydramount (G. T. Gurr), Umělá klovatina (Koh-i-noor, záv. Gama; see BENEŠ 1974), arabic gum (10 g to 15 ml H_2O), dextrin (10 g to 14 ml H_2O), dextran m.w. about 200 000 (10 g to 12 ml H_2O), polyvinylpyrolidone K 25 (Lachema, imported;

* It is hard to say whether this treatment is a "reaction" or a "staining" (in a broader sense). Boths terms will be used here interchangeably.

10 g to 12 ml H_2O), polyvinylalcohol (Sloviol R 20%, Záv. W. Piecka; 1 part to 2 parts of H_2O). To 4 parts of the given medium 1 part of the stock solution of JJK was added. Thus, concerning the final concentration of JJK, all the mounting media tested correspond to the diluted Lugol's solution used for staining. The slides were covered using the given mounting medium immediately after staining *i.e.* as quickly as possible and without any rinse. Then they were kept in the dark. When dry, the cover slides were framed with Canada balsam. With the exception of arabic gum, the staining disappeared more or less quickly in all the JJK containing media tested. If JJK has not been added, the staining fades very soon in all media under study.

To compare the staining of starch in paraffin and frozen sections, the paraffin sections were prepared as given above whereas the frozen ones were obtained as follows (BENEŠ 1973): FAA fixed and 50% ethanol washed objects were brought to 5% ethanol, kept and sectioned there (50 μm) and even the sections were transferred into 5% ethanol. The sectioning was done using the semiconductor freezing microtome Cryotome Tesla with additional knife cooling with solid CO_2. Otherwise, the paraffin and frozen (free floating) sections were stained and handled in the same way as described previously. No differences in staining intensity were seen in the material processed in the two different ways, but colour maintenance was better in paraffin sections.

The following experiment was performed to reveal whether it is possible to keep the material in 50% ethanol unchanged concerning the staining of starch. The objects were fixed as given in the standard procedure. Then they were brought to 50% ethanol, washed and kept there for different time intervals (one day, one week, two weeks). After the definite time period some objects were brought to paraffin. Then, all the objects were sectioned and stained simultaneously. No apparent differences were seen in the objects kept in 50% ethanol for various time spaces.

From the two possibilities of joining JJK and alcian blue staining, the alcian blue/JJK sequence appeared as the more suitable: deparaffinize the slices, rinse in 3% acetic acid, stain 30 min in 0.1% alcian blue (G. T. Gurr) in 3% acetic acid, rinse in 3% acetic acid, then, rinse in H_2O and, thereafter, apply JJK in the standard way. The results are satisfactory. The starch grains are coloured dark violet. The cell walls appear greenish in comparison with the plain alcian blue staining.

The procedure for visualizing starch described here can be used in routine work. The JJK staining remains unchanged for about one year. It has proved useful even in many other plant objects. Since the solutions of JJK become altered within several days due — apparently — to iodine loss, it appears relevant to apply the standard in critical cases *i.e.* slides with paraffin sections of known object processed together with unknown material. The root tip of pea, as given here, may be used for this purpose.

The procedure proved in the present paper seems to be promising for visualizing other polysaccharides detectable by iodine, including those which represent end products of techniques used to reveal transfer or synthetizing enzymes.

Finally, the validity — in general — of the idea of preparing mounting media as given in the mentioned works and in the present one must be noted. In all cases where the final reaction product is not stable enough to obtain

permanent slides in the usual way, the presence in the mounting medium of the reagent used in the final step of the given procedure may be useful. The reasons why just Karo sirup or arabic gum are the relevant mounting media in the present case were not investigated.

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BOOK REVIEW

BRYANT, J. A. (ed.): **Molecular Aspects of Gene Expression in Plants.** — Academic Press, London—New York—San Francisco, 1976, 338 pp., 21 $.

This book was published in the International Series of Monographs devoted to experimental botany as Volume 11. The consulting editor of the monographs is J. F. Sutcliffe, the University of Sussex.

The research on the organization and function of genes and their expression in plants lags behind research on bacteria and animals, especially when the molecular level is considered. Due to the need to improve the yield and quality of crops, more attempts must be made to modify the genetic control of plant cells and to regulate gene expression artificially. This requires a better insight into the biochemistry of plant genes, what is the main object of the reviewed book.

The introductory chapter (written by J. A. Bryant) deals with nuclear DNA (properties, amount, synthesis, repair, turnover) and includes data and methods on DNA extraction, base composition, thermal denaturation, buoyant density *etc.*). This chapter is followed by a section dealing with RNA structures and metabolism (D. Grierson), where data on types of RNA, RNA fractionation, DNA—RNA hybridization, synthesis of ribosomal RNA and regulation of RNA metabolism are presented. Since the gene expression is manifestated by specification of the amino-acid sequence, a chapter written by C. M. Bray is devoted to the synthesis of cellular proteins (amino-acid activation, peptide chain initiation and elongation, chain termination a peptide release). The ability of plants to photosynthesize is an important feature which differentiates them from animals. The hereditary continuity of chloroplasts, containing DNA is a strong evidence that they possess their own genetic system. A special chapter written by J. A. Bryant is thus devoted to the synthesis of nucleic acids and proteins in chloroplasts (and in mitochondria). This chapter includes data on cytoplasmic inheritance. The concluding chapters describe the plant cell cycle and molecular aspects of differentiation (J. A. Bryant) and plant growth substances (A. J. Trewavas). Each chapter is accompanied by "suggestions for further reading" where the most relevant books and articles on the described topic are quoted.

The book is aimed at post-graduates, established scientists and teachers who wish to discover more on the function of plant genes or are contemplating research in this field.

T. GICHNER (*Praha*)